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SODIUM OXY-MERCURY-ORTHO-NITRO PHENOLATE (MERCUROPHEN)

WITH SPECIAL REFERENCE TO ITS PRACTCAL VALUE AS A DISINFECTANT

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WITH THE ASSISTANCE OF
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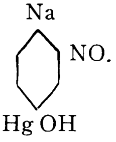
In a preliminary report[1] we briefly described the properties of a mercurial compound, elaborated during a systematic investigation in the chemotherapy of mercurial compounds, which was found to possess certain superior antiseptic and germicidal properties and to which the name of "mercurophen" was given. Since then additional laboratory and clinical studies have been conducted on a large scale and the object of this article is to give in a brief manner a further report on the properties of this compound and its value as a disinfectant. The additional work has confirmed our earlier results and the superiority of mercurophen over other mercurials, notably mercuric chlorid, which was taken as a standard for comparison, and in addition it has been shown that the new compound possesses a marked affinity and destructive influence for staphylococci and spore-forming organisms and also possesses other distinctive features of much interest and importance from the viewpoint of chemotherapy.

Mercurophen has been designated chemically as sodium oxymercuryorthonitrophenolate, containing 53% mercury and having the following structural formula:



The exact position of mercury in the molecule is not definitely demonstrated.

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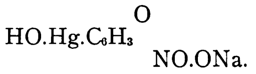
*This work was made possible by funds accruing from the dispensing of arsphenamin.

[1] Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: Jour. Am. Med. Assn., 1917, 68, p. 1458.

It is a brick red powder, freely soluble in hot water, and capable of being molded into tablets, likewise freely soluble; solutions may be prepared as concentrated as 1:100 but not usually beyond this degree. The solutions are deep amber in color, which is distinctive in dilutions as high as 1:10,000 or more. Concentrated solutions, as 1:500 and 1:1,000, after standing several days may show a slight amount of reddish sediment but solutions maintain an even germicidal potency over long periods of time providing evaporation does not occur. The powder and its solutions are odorless; solutions of 1:1,000 have a slightly bitter metallic taste while higher dilutions, as 1:5,000 and 1:10,000, are practically tasteless.

As previously stated mercurophen was prepared during a study and elaboration of compounds of mercury and phenol; when our preliminary report was made we were not aware that this compound had been briefly described by Häntzsch and Auld[2] under the name of "merkurinitrophenole," the sodium salt of mercury hydrate aci-ortho-nitrophenol with a theoretical sodium content of 6.1 and the following structural formula:



$$\mathrm{HO.Hg.C_6H_3}\begin{matrix}\mathrm{O}\\ \mathrm{NO.ONa.}\end{matrix}$$

The authors referred to made no biological studies of this compound, and further references to it have not been found.

THE ANTISEPTIC OR BACTERIOSTATIC PROPERTIES OF MERCUROPHEN

As is well known very slight modifications in technic may alter the results of germicidal tests; for this reason we have sought to employ simple methods along well known lines and to present in this paper the essentials of each method employed.

The antiseptic or bacteriostatic (a term proposed by Basil Gildersleeve) test yields very sharp, decisive and easily read results concerning the power of a substance to inhibit multiplication of a microorganism, although actual killing or permanent destruction and crippling are not determined unless subcultures are made for viable organisms; as a general rule, we have found with B. typhosus and staphylococci that there is a distinct relationship between the bacteriostatic and germicidal values of a substance under study although with spore bearing bacteria as B. anthracis and B. subtilis, a compound may possess marked bacteriostatic and feeble germicidal activity.

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[2] Bericht. Deutsch. Chem. Gesellsch., 1906, 39, p. 1105.

In making these tests dilutions of mercurophen and other germicidal substances were made from stock 1: 1,000 solutions with sterile distilled water and 1 c c of each dilution placed in a series of sterile test tubes; dilutions of phenol were prepared in a similar manner from a standardized 5% solution. The test organism was grown in broth for 48 hours, shaken with beads to break up clumps and 1 c c added to 100 c c of plain neutral beef extract broth of which 5 c c was added to each test tube, making the total volume 6 c c. In this manner we were sure that each tube was seeded with the same number of bacteria, and owing to the simplicity of the technic errors were reduced to a minimum. In each experiment controls of the cultures were included; the results were recorded after 5 days' incubation in a thermostat. In numerous experiments subcultures were made of the contents of the tubes showing no evidence of growth, by transferring a 4 mm. loopful to a slant of plain neutral agar.

TABLE 1

RESULTS OF ANTISEPTIC TESTS WITH B. TYPHOSUS

Substance	Percentage Pure Hg	Final Dilutions and Results									
		1: 300,000	1: 360,000	1: 420,000	1: 480,000	1: 540,000	1: 600,000	1: 1,200,000	1: 1,800,000	1: 2,400,000	1: 3,000,000
Mercurophen No. 15..........	53	—	—	—	+	+	+	+	+	+	+
Mercurophen No. 16..........	47	—	—	+	+	+	+	+	+	+	+
Mercuric chlorid.	74	—	—	—	—	—	+	+	+	+	+
Hg Oxycyanid...	83	—	—	—	—	—	—	—	+	+	+
Hg Cacodylate..	20	+	+	+	+	+	+	+	+	+	+
Hg Succinimid...	50	—	—	—	—	—	+	+	+	+	+

— = no growth; + = growth.

TABLE 2

RESULTS OF ANTISEPTIC TESTS WITH STAPHYLOCOCCUS AUREUS

Substance	Percentage Pure Hg	Final Dilutions and Results									
		1: 300,000	1: 360,000	1: 420,000	1: 480,000	1: 540,000	1: 600,000	1: 1,200,000	1: 1,800,000	1: 2,400,000	1: 3,000,000
Mercurophen No. 15..........	53	—	—	—	—	—	—	—	—	—	+
Mercurophen No. 16..........	47	—	—	—	—	—	—	—	—	+	+
Mercuric chlorid.	74	—	—	—	—	—	+	+	+	+	+
Hg Oxycyanid...	83	—	—	—	—	—	—	+	+	+	+
Hg Cacodylate..	20	+	+	+	+	+	+	+	+	+	+
Hg Succinimid...	50	—	—	+	+	+	+	+	+	+	+

— = no growth; + = growth.

The Rawling strain of B. typhosus was employed; the strain of staphylococcus aureus was obtained from the pus of a furuncle and the strain of B. anthracis from a malignant pustule, about 2 years ago.

The results of several experiments with B. typhosus, staphylococcus aureus and B. anthracis and different lots of mercurophen and a number of well known mercurials are shown in tables 1, 2 and 3;

a summary of the general results are shown in table 4 in which the highest antiseptic or bacteriostatic dilutions are given with a comparison of the mercurial compounds with phenol as expressed in the "phenol coefficients," and a comparison of the mercuorphen and other mercurial compounds with mercuric chlorid, as expressed in the column under "mercuric chlorid coefficients."

TABLE 3

RESULTS OF ANTISEPTIC TESTS WITH B. ANTHRACIS

Substance	Percentage Pure Hg	Final Dilutions and Results									
		1: 300,000	1: 360,000	1: 420,000	1: 480,000	1: 540 000	1: 600,000	1: 1,200,000	1: 1,800,000	1: 2,400,000	1: 3,000,000
Mercurophen No. 15..........	53	—	—	—	—	—	—	—	—	+	+
Mercurophen No. 16..........	47	—	—	—	—	—	—	—	+	+	+
Mercuric chlorid.	74	+	+	+	+	+	+	+	+	+	+
Hg Oxycyanid...	83	+	+	+	+	+	+	+	+	+	+
Hg Cacodylate..	20	+	+	+	+	+	+	+	+	+	+
Hg Succinimid...	50	+	+	+	+	+	+	+	+	+	+

— = no growth; + = growth.

TABLE 4

SUMMARY OF RESULTS OF ANTISEPTIC TESTS; PHENOL AND MERCURIC CHLORID COEFFICIENTS

Substance	Percentage Pure Hg	Highest Antiseptic Dilution after Incubation for 5 Days at 37 C.			Phenol Coefficients			Mercuric Chlorid Coefficients		
		B. typhosus	Staph. aureus	B. anthracis	B. typhosus	Staph. aureus	B. anthracis	B. typhosus	Staph. aureus	B. anthracis
Mercurophen No. 4...........	53	1: 500,000	1:1,200,000	1:1,200,000	714	1714	2500	—0.92	+2.2	+4.3
Mercurophen No. 6...........	53	1: 480,000	1:1,200,000	1:1,000,000	685	1714	2083	—0.9	+2.2	+3.5
Mercurophen No.11..........	53	1: 480,000	1:1,800,000	1:1,400,000	685	2572	2916	—0.9	+3.3	+5.0
Mercurophen No. 12..........	53	1: 420,000	1:1,800,000	1:1,200,000	600	2572	2500	—0.8	+3.3	+4.3
Mercurophen No. 15..........	53	1: 420,000	1:2,400,000	1:1,800,000	600	3428	3666	—0.8	+4.4	+6.4
Mercurophen No. 16..........	47	1: 360,000	1:1,800,000	1:1,200,000	514	2572	2500	—0.66	+3.3	+4.3
Hg Oxycyanid...	83	1:1,200,000	1: 600,000	1: 280,000	1714	857	583	+2.2	+1.1	1.0
Hg Succinimid...	50	1: 540,000	1: 360,000	1: 250,000	771	514	520	1.0	—0.66	—0.9
Hg Cacodylate..	20	1: 180,000	1: 120,000	1: 100,000	257	171	208	—0.33	—0.2	—0.4
Phenol...........	0	1: 700	1: 700	1: 480	0	0	0	0	0	0
Mercuric chlorid.	74	1: 540,000	1: 540,000	1: 280,000	771	771	583	0	0	0

These results may be summarized as follows:

1. The bacteriostatic value of mercuric chlorid and other mercurial compounds for all three of the test organisms bears a direct relationship to their content in mercury and probably to the degree of

electrolytic dissociation. For example, a mercuric chlorid containing 74% mercury proved bacteriostatic for B. typhosus in dilution of 1: 540,000, while the oxycanid of mercury containing 83% mercury proved bacteriostatic in dilutions as high as 1: 1,200,000, the cacodylate—which showed in our analysis but 20% mercury—was bacteriostatic in only 1: 180,000. The same relationship between content of mercury in mercuric chlorid and other mercurials and bacteriostatic activity was shown in experiments with staphylococcus aureus and B. anthracis.

2. Phenol showed the same bacteriostatic activity for both B. typhosus and staphylococcus aureus being about 1:600 and 1:480 for anthrax bacilli and B. subtilis.

3. Mercurophen is more bacteriostatic for staphylococci and anthrax bacilli than mercuric chlorid and other of the ordinary mercury compounds and this constitutes an important observation from the chemotherapeutic and practical standpoint. For example, mercurophen containing 53% mercury was bacteriostatic.for staphylococci in dilutions varying from 1: 1,200,000 to 1: 2,400,000, while mercuric chlorid containing 74% mercury was bacteriostatic in 1: 540,000, and the oxycyanid containing as much as 83% mercury in but 1: 600,000. With the anthrax bacillus a similar superiority of mercurophen was noted inasmuch as this compound was bacteriostatic in about 1: 1,400,000 whereas mercuric chlorid was bacteriostatic in but 1: 280,000.

As shown in table 4 under mercuric chlorid coefficients, mercurophen was somewhat less bacteriostatic for B. typhosus than mercuric chlorid being from —0.8 to —0.92 calculated on the basis of mercuric chlorid as 1.0, while with the Staph. aureus and B. anthracis, mercurophen gave indices varying from + 2.2 to + 6.4 as calculated on the same basis.

4. As compared with phenol, mercurophen was generally from 514 to 714 times more bacteriostatic for B. typhosus while mercuric chlorid was 771 times more bacteriostatic; with Staph. aureus the mercurophens gave coefficiencies varying from 3,428 to 1,714 as compared with 771 shown by mercuric chlorid. With B. anthracis, mercurophen showed a bacteriostatic phenol coefficient of 2,000 to 3,600 as compared to a coefficient of 600 by mercuric chlorid.

RELATION OF ANTISEPTIC TO GERMICIDAL ACTIVITY

As previously stated, the antiseptic values of the substances studied were recorded in this method after a period of incubation for 5 days; the highest antiseptic values were observed at the end of the first 24 hours' incubation but from then to the end of 5 days, growths usually appeared in the next (one or more) of the lower dilutions. With the completion of the 5 day period subcultures of the clear or negative tubes showing antiseptic, restraining or bacteriostatic activity made by transferring two 4 mm. loopfuls of clear broth to slants of agar, usually showed the presence of viable staphylococci and typhoid bacilli in at least several dilutions lower than the bacteriostatic dilution; for example, mercurophen proved antiseptic for B. typhosus in dilution of 1:600,000 and germicidal in 1:360,000; mercuric chlorid was antiseptic in dilution of 1:540,000 and germicidal in 1:420,000; phenol was antiseptic in dilution of 1:660 and germicidal in 1:540. With B. anthracis and B. subtilis the differences were more striking; for example, phenol proved antiseptic for B. subtilis in dilution of 1:480 but germicidal in less than 1:60. With the exception of the spore-forming bacilli, there was found with this technic a definite relation between the antiseptic and germicidal values of the substances under study, and since the technic of the antiseptic test is simple and the results sharp and easily read, we have adopted this technic for determining comparative antibacterial properties of substances in the course of our chemotherapeutic studies.

TABLE 5

GERMICIDAL ACTIVITY OF PHENOL AND MERCUROPHEN FOR B. TYPHOSUS IN THE HYGIENIC LABORATORY METHOD

Substance	Dilution	Time Culture Was Exposed to Action of Disinfection in Minutes						Phenol Coefficient
		2½	5	7½	10	12½	15	
Phenol	1:70	—	—	—	—	—	—	
	1:80	+	+	—	—	—	—	70 | 40,000
	1:90	+	+	+	—	—	—	
	1:100	+	+	+	+	+	+	= 571.4
	1:110	+	+	+	+	+	+	
Mercurophen No. 12 Hg 53%	1:40,000	—	—	—	—	—	—	90 | 120,000
	1:60,000	+	—	—	—	—	—	= 1333.3
	1:80,000	+	+	—	—	—	—	
	1:100,000	+	+	+	+	—	—	2 | 1904.7
	1:120,000	+	+	+	+	+	—	
	1:160,000	+	+	+	+	+	+	952.3

Beef infusion broth +0.5.

TABLE 6

RESULTS OF GERMICIDAL TESTS WITH B. TYPHOSUS BY THE HYGIENIC LABORATORY METHOD

Substance	Dilution	Time Culture Was Exposed to Action of Disinfection in Minutes						Phenol Coefficient
		2½	5	7½	10	12½	15	
Mercurophen No. 16 Hg 47%	1:30,000	—	—	—	—	—	—	70 | 30,000 = 428.5 90 | 60,000 = 666.6 2 | 1105.1 552.5
	1:40,000	+	+	—	—	—	—	
	1:50,000	+	+	—	+	—	—	
	1:60,000	+	+	+	+	+	—	
	1:80,000	+	+	+	+	+	+	
	1:100,000	+	+	+	+	+	+	
Mercurophen No. 6 Hg 53%	1:40,000	—	—	—	—	—	—	70 | 40,000 = 571.4 90 | 160,000 = 1777.7 2 | 2349.1 1174.5
	1:60,000	+	—	—	—	—	—	
	1:80.000	+	+	—	—	—	—	
	1:100,000	+	+	+	+	—	—	
	1:120,000	+	+	+	+	+	—	
	1:160,000	+	+	+	+	+	—	
Mercuric Chlorid Hg 74%	1:40,000	—	—	—	—	—	—	70 | 40,000 = 571.4 90 | 100,000 = 1111.1 2 | 1682.5 841.2
	1:60,000	+	—	—	—	—	—	
	1:80,000	+	—	+	—	—	—	
	1:100,000	+	+	+	+	+	—	
	1:120,000	+	+	+	+	+	+	
	1:160,000	+	+	+	+	+	+	
Mercury Benzoate Hg 45%	1:30,000	—	—	—	—	—	—	70 | 30,000 = 428.5 90 | 80,000 = 888.8 2 | 1317.3 658.6
	1:40,000	+	—	—	—	—	—	
	1:60,000	+	+	+	—	—	—	
	1:80,000	+	+	+	—	+	—	
	1:100,000	+	+	+	+	+	+	
	1:120,000	+	+	+	+	+	+	
Mercury Cacodylate Hg 20%	1:10,000	—	—	—	—	—	—	70 | 10,000 = 142.8 90 | 30,000 = 333.3 2 | 476.1 238.0
	1:20,000	+	+	—	—	—	—	
	1:30,000	+	+	—	+	—	—	
	1:40,000	+	+	+	+	+	—	
	1:60,000	+	+	+	+	+	+	
	1:80,000	+	+	+	+	+	+	
Mercury Sozoidolate Hg 32%	1:20,000	—	—	—	—	—	—	70 | 20,000 = 285.7 90 | 60,000 = 666.6 2 | 952.3 **476.1**
	1:30,000	+	+	—	—	—	—	
	1:40,000	+	+	—	+	—	—	
	1:60,000	+	+	+	+	+	—	
	1:80,000	+	—	+	+	+	+	
	1:100,000	+	+	+	+	+	+	

Plain neutral beef extract broth.

THE GERMICIDAL PROPERTIES OF MERCUROPHEN AS DETERMINED BY THE HYGIENIC LABORATORY METHOD

In conducting these tests the Rawling strain of B. typhosus was employed and the technic was exactly as described by Anderson and McClintic[3] except that the beef infusion broth employed had an end reaction of + 0.5 instead of + 1.5, inasmuch as broth with an end reaction of + 1.5 did not yield good growths of typhoid bacilli. Parallel tests were made in exactly the same manner with beef extract broth neutral to phenolphthalein inasmuch as this medium was previously employed by us in the Rideal-Walker tests. The dilutions of phenol were prepared from a 5% solution obtained from the Hygienic Laboratory and also from our own standardized solution varying not more than 0.03% from the standard 5% solution. Subcultures were made with the regulation 4 mm. loop standardized in the Hygienic Laboratory.

TABLE 7

SUMMARY OF RESULTS OF GERMICIDAL TESTS WITH B. TYPHOSUS IN THE HYGIENIC LABORATORY METHOD WITH BEEF INFUSION BROTH

Substance	Highest Germicidal Dilution		Phenol Coefficient	Mercuric Chlorid Coefficient
	2½ Minutes	15 Minutes		
Phenol	1:70	1:90		
Mercurophen No. 4	1:40,000	1:200,000	1396	+1.5
Mercurophen No. 6	1:40,000	1:160,000	1174	+1.4
Mercurophen No. 11	1:40,000	1:120,000	952	+1.1
Mercurophen No. 12	1:40,000	1:120,000	952	+1.1
Mercury benzoate	1:30,000	1:80,000	658	—0.78
Mercury zozoiodolate	1:20,000	1:60,000	476	—0.56
Mercury cacodylate	1:10,000	1:30,000	238	—0.275
Mercuric chlorid	1:40,000	1:100,000	841	

TABLE 8

SUMMARY OF RESULTS OF GERMICIDAL TESTS WITH B. TYPHOSUS IN THE HYGIENIC LABORATORY METHOD WITH BEEF EXTRACT BROTH

Substance	Highest Germicidal Dilution		Phenol Coefficient	Mercuric Chlorid Coefficient
	2½ Minutes	15 Minutes		
Phenol	1:70	1:90		
Mercurophen No. 4	1:40,000	1:160,000	1174	+1.5
Mercurophen No. 6	1:40,000	1:160,000	1174	+1.5
Mercurophen No. 11	1:40,000	1:120,000	952	+1.25
Mercurophen No. 12	1:40,000	1:120,000	952	+1.25
Mercury benzoate	1:30,000	1:80,000	658	—0.87
Mercury zozoiodolate	1:20,000	1:60,000	476	—0.65
Mercury cacodylate	1:10,000	1:30,000	237	—0.31
Mercuric chlorid	1:40,000	1:80,000	729	

The results of a series of tests are shown in tables 5 and 6 and summarized in tables 7 and 8, the latter comparing the results observed with a beef infusion broth of + 0.5 and reaction to those observed with neutral beef extract broth.

These results may be summarized as follows:

1. The phenol coefficiency of mercurophen varied from 900 to 1,300 while that of mercuric chlorid was about 840.

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[3] Jour. Infect. Dis., 1911, 8, p. 1.

2. As shown in table 6 the phenol coefficiency of mercurophen and the common mercurial compounds bore some relationship to the percentage of pure mercury. A mercurophen having 53% pure mercury showed a coefficiency of 1,174 while a second showing on analysis about 47% mercury, had a coefficiency of but 552. The results with mercury benzoate, mercury cacodylate and mercury zozoiodolate all having varying percentages of mercury, show a similar bearing of mercury content on the results of these germicidal tests as was found with the antiseptic tests previously described.

3. As a general rule, mercurophen and mercuric chlorid killed B. typhosus in this test in a 1:400,000 dilution in 2½ minutes, but in the longer period of exposure—namely, 15 minutes—mercurophen proved superior inasmuch as dilutions varying from 1:120,000 to 1:200,000 were germicidal while mercuric chlorid was germicidal in dilutions varying from 1:80,000 to 1:100,000; the higher phenol coefficiencies of mercurophen were largely due to this property of killing B. typhosus more quickly than mercuric chlorid and the other compounds.

This advantage of mercurophen is shown in the column of mercuric chlorid coefficiencies in tables 7 and 8, where mercuric chlorid is taken as 1.0 and the mercurophens and other compounds expressed accordingly. As previously stated in the antiseptic tests mercuric chlorid proved equal to or slightly superior to mercurophen; the differences are very probably to be ascribed to differences in the rapidity of action, mercurophen being somewhat more rapid in its typhoidicidal activity, as discussed below and shown in table 9.

Aside from this advantage mercurophen was more germicidal than expected inasmuch as it contains but 53% mercury as compared with 74% mercury in mercuric chlorid; this superiority is due to the peculiar constitution of the molecule of mercurophen, and constitutes an observation of great interest and importance from the viewpoint of chemotherapy.

4. As shown in tables 7 and 8 but very slight differences in the results were observed with beef infusion broth of + 0.5 and neutral beef extract broth; at times the former medium showed slightly higher values as will be noted in the tables with mercurophen No. 4 and mercuric chlorid.

These results vary considerably from those previously reported with the Rideal-Walker method and we are at a loss to explain the discrepancies; mercurophen is not as germicidal by the Hygienic Laboratory method as we originally believed. In the former work a strain of B. typhosus was employed which was isolated over 20 years ago and has been widely used in the agglutination tests; comparative tests with the Rawling's strain showed that the former grew more profusely in the same broth and is somewhat more difficult to kill but these differences do not explain all of the discrepancy. The phenol previously employed had been standardized about 2 months before the tests were made and inasmuch as phenol kills B. typhosus in dilution of 1:70 to 1:90 in 2½ to 15 minutes whereas our former phenol required 1:20 to 1:40 dilutions to kill the former strain of B. typhosus, it is likely that a large part of the error was due to defective stock solution of phenol; unfortunately none was available for further tests to settle this supposition in a more definite manner.

THE RAPIDITY OF GERMICIDAL ACTIVITY OF MERCUROPHEN AND OTHER MERCURIAL COMPOUNDS

Further evidence of the more rapid germicidal activity of mercurophen as compared with mercuric chlorid, is shown in the results of the following experiment:

In a series of test tubes were placed 10 c c of varying dilutions of the mercurial compounds in distilled water seeded with 0.1 c c of 24-hour broth cultures of the test micro-organism; the controls were prepared with sterile distilled water and culture alone. After intervals of 15, 30, 60 and 120 minutes at room temperature 0.1 c c of each tube was plated with 10 c c of agar at 45 C. and the plates counted after 48 hours' incubation; the results of one experiment shown in table 9 indicate that mercurophen is more rapidly germicidal for staphylococci and typhoid bacilli than equal strength dilutions of mercuric chlorid; for example, a 1:100,000 dilution of mercurophen was completely germicidal for staphylococci after an exposure of 1 hour and for typhoid bacilli in 15 minutes whereas mercuric chlorid failed to destroy all staphylococci in 2 hours and required 60 minutes for the complete destruction of the typhoid bacilli.

TABLE 9

THE RAPIDITY OF GERMICIDAL ACTIVITY OF MERCUROPHEN AND MERCURIC CHLORID

Substances	Dilution	Action on Staphylococci				Action on B. typhosus			
		15 Min.	30 Min.	60 Min.	120 Min.	15 Min.	30 Min.	60 Min.	120 Min.
Mercurophen........	1:100,000	200	152	sterile	sterile	sterile	sterile	sterile	sterile
Mercuric chlorid.....	1:100,000	520	230	20	18	600	150	sterile	sterile
Mercurophen........	1:500,000	unc.	unc.	unc.	2000	230	40	sterile	sterile
Mercuric chlorid.....	1:500,000	unc.	unc.	unc.	530	unc.	230	6	sterile
Mercurophen........	1:1,000,000	unc.	unc.	unc.	unc.	unc.	unc.	unc.	unc.
Mercuric chlorid.....	1:1,000,000	unc.	unc.	unc.	unc.	unc.	unc.	unc.	unc.
Controls............	0	unc.	unc.	unc.	unc.	unc.	unc.	unc.	unc.

The numbers give the colonies per plate; unc. = an uncountable number of colonies.

THE STABILITY OF SOLUTIONS OF MERCUROPHEN

Solutions of mercurophen in pure distilled water kept in tightly stoppered bottles to prevent evaporation do not undergo change in germicidal activity over long periods of time; the same was found true of solutions of mercuric chlorid kept under the same conditions (table 10). Exposure to light has no perceptible effect and solutions are easily kept at ordinary room temperature without any special precautions in regard to the container except the prevention of evaporation. The result of a single experiment bearing on the bacteriostatic activity of solutions of mercurophen and mercuric chlorid for the same strain of staphylococcus aureus over a period of 6 weeks, are shown in table 10. In this experiment both stock 1:1,000 solutions were kept in the laboratory near a window with abundant sunlight, in glass stoppered cylinders; the slight variations in bacteriostatic or antiseptic activity noted over this period of time are readily explained on the basis of experimental error and particularly to variations in the resistance of the strain of staphylococcus; the same culture medium was used throughout.

TABLE 10

THE STABILITY OF MERCUROPHEN AND MERCURIC CHLORID; HIGHEST ANTISEPTIC DILUTION FOR STAPHYLOCOCCUS AUREUS

Age of Stock Solutions	Mercurophen	Mercuric Chlorid
Fresh	1:1,800,000	1:540,000
1 week	1:1,800,000	1:540,000
2 weeks	1:1,200,000	1:480,000
3 weeks	1:1,800,000	1:540,000
4 weeks	1:1,800,000	1:480,000
5 weeks	1:1,200,000	1:480,000
6 weeks	1:1,200,000	1:480,000

THE GERMICIDAL ACTIVITY OF MERCUROPHEN IN BLOOD, ASCITES FLUID AND SERUM

In a menstruum rich in serum proteins the germicidal activity of mercurophen is reduced as compared with similar tests in which serum proteins are absent, but as stated in our preliminary report, the reduction in germicidal activity is not nearly so marked as that occurring with mercuric chlorid and other ordinary mercurial compounds, and with phenol; indeed, this is a distinguishing property of mercurophen as compared with most other mercurial compounds; a probable explanation lies in the fact that mercurophen is remarkably free of precipitating properties, as is shown in experiments summarized in table 13.

Germicidal Activity of Mercurophen in Blood.—In a series of experiments sterile oxalated rabbit and rat blood were employed; 20 c c of each were heavily infected with the addition of 2 c c of a 48-hour broth culture of Staph. aureus and incubated for an hour. In a series of small sterile test tubes equal parts of infected blood were mixed with solutions of mercurophen as 1:500 and 1:1,000, the final dilutions of mercurophen being 1:1,000 and 1:2,000 in a 50% oxalated blood, and allowed to stand at room temperature. At varying intervals 0.1 c c of each mixture was seeded in tubes of broth containing 10 c c or plated with 10 c c of agar at 45 C. and examined after 72 hours' incubation. The results are briefly summarized in tables 11 and 12.

TABLE 11

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL IN OXALATED BLOOD

Substance	Final Dilution in 50 per Cent. Blood	Exposure for Minutes					
		1	5	10	15	30	60
Mercurophen	1:1,000	—	—	—	—	—	—
Mercurophen	1:2,000	+	+	+	+	+	—
Mercuric chlorid	1:1,000	—	—	—	—	—	—
Mercuric chlorid	1:2,000	+	+	+	+	+	+
Phenol	1:40	+	+	+	—	—	—
Phenol	1:80	+	+	+	+	+	—
Salt solution control	....	+	+	+	+	+	+

— = sterile; + = growth.

TABLE 12

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL IN ASCITES FLUID

Substance	Final Dilution in 50 per Cent. Ascites Fluid	Exposure for Minutes					
		1	5	10	15	30	60
Mercurophen	1:2,000	—	—	—	—	—	—
Mercurophen	1:4,000	—	—	—	—	—	—
Mercuric chlorid	1:2,000	—	—	—	—	—	—
Mercuric chlorid	1:4,000	+	+	+	—	—	—
Phenol	1:80	—	—	—	—	—	—
Phenol	1:20	+	+	+	+	—	—
Salt solution control	....	+	+	+	+	+	+

— = sterile; + = growth.

As shown in table 11, a final dilution of 1:1,000 mercurophen in 50% blood was completely germicidal in 1 minute, whereas mercuric chlorid was not germicidal in 5 minutes; in 1:2,000 dilution mercurophen required 60 minutes to sterilize the blood, whereas mercuric chlorid failed in this period and indeed after 2 hours' exposure. Phenol in final dilution of 1:40 required 15 minutes, and in 1:80 required 60 minutes to bring about complete sterilization.

Hemolytic Activity of Mercurophen.—In this connection mention may be made of the results of experiments bearing on the hemolytic activity of solutions of mercurophen, mercuric chlorid and other mercurial compounds in physiologic salt solution, for the washed erythrocytes of various mammals; mercurophen proved hemolytic for washed human erythrocytes in final dilutions 1:16,000 and mercuric chlorid as high as 1:160,000. For sheep cells mercurophen was hemolytic 1:80,000 and mercuric chlorid 1:160,000.

Germicidal Activity of Mercurophen in Ascites Fluid.—With 50% ascites fluid mercurophen in final dilution of 1:4,000 brought about complete sterilization within 1 minute, whereas mercuric chlorid failed in 10 minutes; phenol in final dilution of 1:120 required 30 minutes.

Germicidal Activity of Mercurophen in Serum.—These experiments were conducted at room temperature by mixing in sterile test tubes 2.5 c c of fresh, sterile unheated human serum with 2.5 c c of water solutions of the respective mercurial compounds and seeding the tubes with 0.1 c c of a paper filtered 24-hour broth culture of Staph. aureus. At varying intervals 0.1 c c of each mixture was plated in 10 c c of plain neutral agar at 45 C. and the plates counted at the end of 48 hours. The results shown in table 13 indicate the general superiority of mercurophen in a serum menstruum over other mercurial compounds; for example, mercurophen in final dilution of 1:10,000 proved germicidal after an exposure of 1 minute, whereas mercuric chlorid and the oxycyanid in equal dilutions required over 30 minutes to produce complete sterilization and the succinimid and cacodylate failed even after 2 hours in final dilution as low as 1:2,000.

TABLE 13

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND OTHER MERCURIAL COMPOUNDS IN 50% DILUTION OF SERUM FOR STAPHYLOCOCCUS AUREUS

Compound	Percent. Pure Mercury	Precipitation of Serum Proteins	Final Dilution in 50% Serum	Results after Exposure for Minutes				
				1	15	30	60	120
Mercurophen.........	53	Neg. 1:200	1:50,000	3500	1000	410	71	10
Mercurophen.........	53	Neg. 1:200	1:10,000	sterile	sterile	sterile	sterile	sterile
Mercuric chlorid.....	74	Pos. 1:1,000	1:50,000	unc.	unc.	unc.	unc.	unc.
Mercuric chlorid.....	74	Pos. 1:1,000	1:10,000	unc.	1990	100	sterile	sterile
Hg oxycyanid.......	83	Pos. 1:400	1:50,000	unc.	unc.	unc.	unc.	unc.
Hg oxycyanid.......	83	Pos. 1:400	1:10,000	unc.	1700	300	sterile	sterile
Hg succinimid.......	50	Neg. 1:200	1:50,000	unc.	unc.	unc.	unc.	unc.
Hg succinimid.......	50	Neg. 1:200	1:2,000	unc.	unc.	unc.	unc.	unc.
Hg cacodylate.......	20	Pos. 1:300	1:2,000	unc.	unc.	unc.	unc.	unc.
Control..............	0	....	0	unc.	0	0	0	unc.

Furthermore, the precipitation or coagulation of serum proteins by mercurial compounds does not necessarily parallel the degree of inhibition of germicidal activity of these substances in serum. For example, mercurophen and mercury succinimid are organic compounds containing 53 and 50% pure mercury, respectively, and neither precipitates protein in dilutions as low as 1:200, but the succinimid is over 25 times less germicidal in serum; mercuric chlorid precipitates serum proteins in final dilution as high as 1:1,000 with equal parts of serum, but is equal in germicidal activity to the oxycyanid which precipitates protein only in final dilutions as low as 1:400.

While the technic of these tests does not permit comparison with the results of our former tests conducted after the pipet method of Wright, the general conclusion remains unaltered regarding the superior germicidal activity of mercurophen in a medium rich in proteins as uncoagulated blood, ascites fluid and serum.

THE INFLUENCE OF MERCUROPHEN ON SERUM PROTEINS

Solutions of mercurophen are practically devoid of all precipitating influence on serums, and in view of its germicidal activity this property is highly desirable. With hot water a 1:100 solution of mercurophen may be prepared and a mixture of equal parts of this solution and fresh human serum may show slight clouding but no precipitation, even after standing 24 hours; higher dilutions have no visible effect at all on serum.

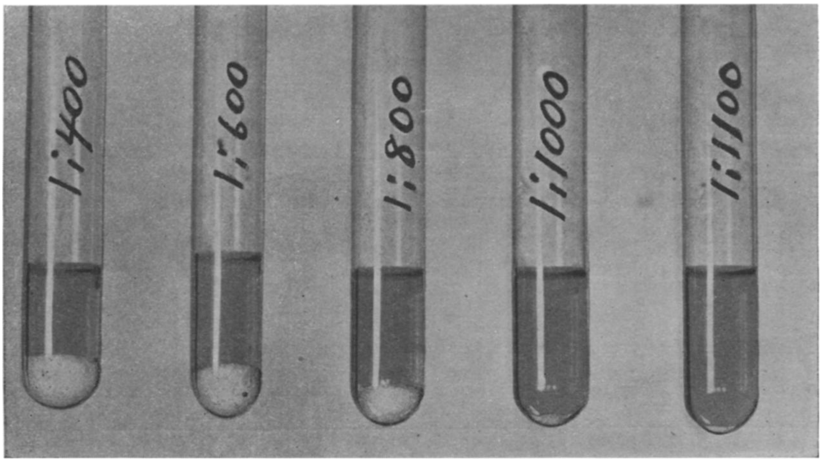

Fig. 1.—The precipitation of serum proteins by mercuric chlorid.

Studies made with other mercurial compounds have shown that this precipitation or coagulation of serum proteins bears no relation to the content in mercury but depends on the nature of the compound, that is, whether organic or inorganic, and the strength of the acid from which a particular salt is derived. For example, mercuric chlorid and mercury sulphate are inorganic compounds and precipitate serum more actively and in higher dilutions than such mercury salts of organic acids as the acetate, oxycyanid and cacodylate of mercury (table 14), although both of the inorganic compounds contain less mercury than one of the salts of an organic acid, namely, the oxycyanid. Furthermore, mercuric chlorid containing 74% mercury produces less precipitate than the sulphate containing 67% and of the salts of organic acids, the oxycyanid containing 83% mercury is hardly more active in precipitating proteins than the cacodylate with but 20% mercury.

Of the two compounds studied only two, namely, mercurophen and mercury succinimid, were found without any effect on serum in dilutions as low as 1:200, and in view of its high germicidal activity the former is rendered peculiarly valuable for the practical disinfection of substances relatively rich in precipitable protein (Figs. 1 and 2).

TABLE 14

THE PRECIPITATION OF HUMAN SERUM PROTEINS BY MERCUROPHEN, MERCURIC CHLORID AND OTHER MERCURIAL COMPOUNDS

Substances	Nature of Compound	Percentage of Pure Hg	Final Dilutions							
			1:200	1:300	1:400	1:600	1:800	1:1,000	1:1,200	1:1,600
Hg bichlorid..........	Inorganic salt	74	4+	4+	4+	4+	2+	+	—	—
Hg sulphate..........	Inorganic salt	67	+	+	+	+	+	+		—
Hg acetate............	Organic salt	63	3+	+	—	—	—	—	—	—
Hg oxycyanid........	Organic salt	83	3+	+	+	—	—	—	—	—
Hg cacodylate........	Organic salt	20	2+	—	—	—	—	—	—	—
Hg succinimid........	Pseudo-complex organic salt	50	—	—	—	—	—	—	—	—
Mercurophen..........	Half-complex organic salt	53	—	—	—	—	—	—	—	—

— = no precipitate; 4+ = very heavy precipitate after standing 24 hours.

In preparing a 1:100 solution of mercury sulphate it was necessary to add 1% sulphuric acid to the water; equal parts of this diluted acid and serum produced no precipitation. All tubes containing dilutions of mercury sulphate showed marked clouding with but slight amounts of precipitate, due probably to solution of the precipitate albumin in the acid fluid.

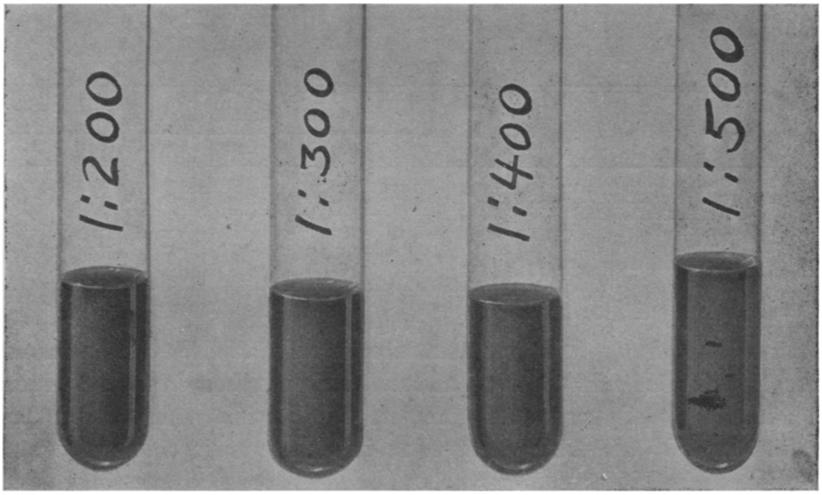

Fig. 2.—The nonprecipitable influence of mercurophen on serum proteins.

RESULTS OF TESTS BEARING ON THE PRACTICAL DISINFECTING PROPERTIES OF MERCUROPHEN

Although mercurial compounds as a group have largely fallen into disuse in favor of formalin and derivatives of coal tar, despite the high germicidal activity of many of them as disinfectants for the skin and such discharges as urine, feces and sputum and the sterilization of instruments, we have extended our studies with mercurophen in a series of experiments to determine its practical disinfecting power, particularly in view of the fact that it does not precipitate protein and maintains a high degree of germicidal activity in the presence of a rich protein medium.

Disinfection of Urine.—Tests have been conducted with urine containing typhoid bacilli alone and with urine as ordinarily voided and collected and containing large numbers of various bacteria. In conducting the experiments equal parts of urine and mercurophen solution were mixed, allowed to stand at laboratory temperature and 0.1 c c plated in 10 c c of agar at 45 C. at varying intervals, the plates being examined after 72 hours' incubation. Mercuric chlorid, phenol, and salt solution controls were included in each experiment.

The results may be expressed as follows:

1. With typhoid urine of weakly acid reaction and albumin free, mercurophen always produced complete sterilization within 5 minutes in final dilution as high as 1:8,000; mercuric chlorid required about 30 minutes in a similar dilution and phenol about 15 minutes in dilution of 1:80 (table 15).

TABLE 15

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL FOR B. TYPHOSUS IN URINE

Substance	Final Dilutions	Results after Exposure for Minutes					
		5	10	15	30	45	60
Mercurophen	1:4,000	—	—	—	—	—	—
Mercurophen	1:8,000	—	—	—	—	—	—
Mercuric chlorid	1:4,000	—	—	—	—	—	—
Mercuric chlorid	1:8,000	+	+	+	—	—	—
Phenol	1:80	+	+	—	—	—	—
Salt solution control	....	+	+	+	+	+	+

— = sterile; + = growth.

2. With ordinary urine of neutral or weakly acid reaction, albumin free but clouded with various bacteria, mercurophen in final dilution of 1:2,000 usually produced complete sterilization within 5 minutes while a 1:4,000 dilution required an hour; mercuric chlorid in final dilution of 1:2,000 required an hour while a 1:4,000 dilution failed. Phenol in final dilution of 1:80 required about 45 minutes (table 16).

TABLE 16

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL FOR THE BACTERIA PRESENT IN URINE AS ORDINARILY COLLECTED

Substance	Final Dilutions	Results after Exposure for Minutes					
		5	10	15	30	45	60
Mercurophen	1:2,000	—	—	—	—	—	—
Mercurophen	1:4,000	+	+	+	+	+	—
Mercuric chlorid	1:2,000	+	+	+	+	+	—
Mercuric chlorid	1:4,000	+	+	+	+	+	+
Phenol	1:80	+	+	+	+	—	—
Salt solution control	....	+	+	+	+	+	+

— = sterile; + = growth.

Most interest concerns the disinfection of the urine of persons suffering with typhoid fever, and mercurophen in final dilution of 1: 5,000 secured by mixing 1 part of a 1: 1,000 solution of mercurophen with 4 parts of urine may be relied on to accomplish this purpose with an exposure of at least 5 minutes at ordinary room temperature.

Disinfection of Feces.—As stated in our previous communication, mercurophen in final dilution of 1: 5,000 with an emulsion of feces made by shaking 1 gm. in 100 c c of sterile distilled water, produced complete sterilization at room temperature in a period of 30 minutes or less; with mercuric chlorid a dilution of 1: 2,000 was required for similar results.

Inasmuch as this emulsion of feces was more dilute than usually dealt with in actual practice we have conducted a series of experiments consisting in adding to fresh semi-solid feces an equal weight of different solutions of mercurophen, mercuric chlorid and phenol; as a general rule an equal weight of the disinfectant was in volume closely parallel by rough measurement, to the volume of feces.

After the addition of the disinfectant solution to the feces the material was thoroughly broken up, but it was not always possible to avoid numerous clumps of feces large enough to protect bacteria within them. After standing at room temperature for varying intervals of time, one 4 mm. loopful of each mixture was plated with 10 c c of agar at 45 C. and the plates counted after 48 hours' incubation.

The results were irregular depending largely on the consistency of the feces and the thoroughness of mixture with the disinfectants. With diarrheal stools and those secured after brisk catharsis with salines, the results were more regular and the material more easily sterilized than the case with the normal semi-solid feces.

TABLE 17

THE DISINFECTION OF FECES BY MERCUROPHEN, MERCURIC CHLORID AND PHENOL

Substances	Final Dilution	Results after Exposure for Minutes				
		10	20	30	45	60
Mercurophen	1:1,000	—	—	—	—	—
Mercurophen	1:2,000	unc.	380	290	150	—
Mercuric chlorid	1:1,000	few	—	—	—	—
Mercuric chlorid	1:2,000	unc	unc.	unc.	420	140
Phenol	1:40	unc.	unc.	400	150	80

— = sterile; unc. = an uncountable number of colonies.

The results of one experiment with semi-solid feces are shown in table 17; in this experiment equal weights of feces and 1: 500 and 1: 1,000 solutions of mercurophen and mercuric chlorid and 1: 20 phenol were mixed and plated at intervals varying from 10-60 minutes.

The results are fairly representative of the series of experiments and show that a final dilution of mercurophen 1: 1,000 produces sterilization within 10 minutes, while a 1: 2,000 dilution requires at least 1 hour (table 17); similar results were observed with mercuric chlorid although inferior to mercurophen, whereas phenol in 1: 40 failed to produce complete sterilization within the hour.

Examination of the plates usually showed a predominating number of spore-forming bacteria; gram-negative bacilli of the typhoid-colon group were generally absent, and we feel quite certain that the addition to the semi-solid typhoid stool of an equal volume of 1: 1,000 solution of mercurophen will result in the complete destruction of typhoid bacilli within a period of 1 hour at room temepratures.

Disinfection of Sputum.—Experiments on the disinfection of sputum consisted in thoroughly mixing equal parts of sputum with varying dilutions of the disinfectant and plating 0.1 c c of each mixture with 10 c c of agar at 45 C. after varying intervals of exposure at room temperature.

The results of one experiment conducted with a thin sanguineous sputum of pneumonia complicating influenza and containing pneumococci, nonhemolytic streptococci and other organisms is shown in table 18; the results of this and similar experiments have shown that the mixture of equal parts of sputum and 1: 2,000 mercurophen (final 1: 4,000 dilution) results in sterilization within a few minutes, whereas mercuric chlorid failed to sterilize in this dilution in periods of 30 minutes and required 15 minutes to produce sterilization with a 1: 2,000 dilution.

TABLE 18

THE DISINFECTION OF SPUTUM BY MERCUROPHEN, MERCURIC CHLORID AND PHENOL

Substance	Final Dilutions	Results after Exposure for Minutes					
		1	3	5	10	15	30
Mercurophen....................	1:2,000	—	—	—	—	—	—
Mercurophen....................	1:4,000	—	—	—	—	—	—
Mercuric chlorid................	1:2,000	+	+	+	+	—	—
Mercuric chlorid................	1:4,000	+	+	+	+	+	+
Phenol...........................	1:40	+	+	+	+	+	—
Salt solution control...........	....	+	+	+	+	+	+

— = sterile; + = growth.

With thick gelatinous sputa of lobar pneumonia and the caseous sputum of tuberculous persons, stronger solutions of mercurophen were required for sterilization, but as a general rule the addition of an equal part of 1: 1,000 solution of mercurophen to such sputa resulted in complete sterilization within an hour except in the case of large caseous or gelatinous masses of sputum which resisted for that time permeation of the disinfectant.

The Disinfection of Catheters.—Experiments on the sterilization of rubber catheters were conducted with ordinary rubber tubing cut into pieces of 1 inch length, soaked for an hour in a broth culture of Staph. aureus and dried in the air. A number of such pieces of tubing were placed in beakers containing 1:5,000 solution of mercurophen and mercuric chlorid and 1:40 phenol and kept at room temperature; after varying intervals of exposure pieces of tubing were removed from the several disinfectants, washed with sterile water and planted in flasks of glucose broth. Controls were conducted in the same manner with normal salt solution. After 48 hours' incubation the results were recorded and those shown in table 19 are representative of the results observed in a series of tests.

TABLE 19

RESULTS OF DISINFECTION TESTS EMPLOYING INFECTED RUBBER TUBING AND SOLUTIONS OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL

Substance	Dilution	Exposure for Minutes						
		1	3	5	8	10	15	20
Phenol	1:40	+	+	+	+	+	+	+
Mercuric chlorid	1:5,000	+	+	+	+	—	—	—
Mercurophen	1:5,000	+	+	+	—	—	—	—
Salt solution control	—	+	+	+	+	+	+	+

+ = growth; — = sterile.

As shown in table 19 mercurophen in dilution of 1:5,000 usually produced complete sterilization with an exposure of 8 minutes, whereas mercuric chlorid required 10 minutes or more and phenol in 1:40 dilution failed to sterilize even after 20 minutes' exposure. With tubing heavily infected with spore-bearing bacilli like B. subtilis, much stronger dilutions of both mercurophen and mercuric chlorid were required as 1:500 solutions with periods of exposure approximating 30 minutes.

The results of these experiments have shown that catheters which cannot be readily sterilized with heat, may be sterilized with a 1:5,000 dilution of mercurophen in an exposure of at least 10 minutes.

Disinfection of Rubber Gloves.—Experiments conducted with surgeons' gloves contaminated with staphylococci have shown that immersion in a warm solution of mercurophen 1:5,000 results in sterilization within a period of 1 minute exposure, whereas mercuric chlorid requires an exposure of 1-3 minutes; in 1:2,000 dilution both mercurophen and mercuric chlorid bring about sterilization within a minute.

Disinfection of Instruments.—Experiments on the disinfection of metal instruments were conducted by contaminating scissor blades, scalpels and hemostatic forceps by immersion in broth cultures of Staph. aureus followed by drying in the air. These instruments were then placed in beakers containiny varying dilutions of the disinfectant, and normal salt solution as a control and after varying intervals of exposure at room temperature was removed with sterile forceps, washed with sterile water to remove disinfectant and cultured by placing each in a large flask of broth. The results observed with one experiment are shown in table 20; the controls invariably showed heavy growths.

TABLE 20

THE DISINFECTION OF INSTRUMENTS BY MERCUROPHEN, MERCURIC CHLORID AND PHENOL

Substance	Final Dilution	Duration of Exposure for Minutes			
		1	5	10	15
Mercurophen	1:5,000	—	—	—	—
Mercurophen	1:10,000	+	—	—	—
Mercuric chlorid	1:5,000	—	—	—	—
Mercuric chlorid	1:10,000	+	+	+	+
Phenol	1:40	+	+	+	+

— = sterile; + = growth.

Mercurophen and mercuric chlorid in dilution of 1:5,000 usually disinfected such instruments after an exposure of 1 minute; in 1:10,000 dilution mercurophen required from 1-5 minutes while mercuric chlorid in equal strength usually failed to disinfect in a period of 15 minutes. Phenol in dilution of 1:40 likewise failed to disinfect these instruments within the 15 minute period of exposure.

In a second series of experiments instruments were picked up without previous preparation, dropped into flasks containing equal parts of nutrient broth and disinfectant solution and incubated for 48-72 hours. With this technic mercurophen in final dilution as high as 1:15,000 proved antiseptic, while mercuric chlorid was antiseptic in 1:3,000 and phenol in 1:40 to 1:60 (table 21).

TABLE 21

THE DISINFECTION OF INSTRUMENTS BY MERCUROPHEN, MERCURIC CHLORID AND PHENOL

Substance	Final Dilutions and Results							
	1: 2,000	1: 4.000	1: 6.000	1: 8,000	1: 10,000	1: 12,000	1: 15,000	1: 18,000
Mercurophen	—	—	—	—	—	—	—	+
Mercuric chlorid	—	—	—	—	+	+	+	+
Phenol	1:40 —	1:80 +	1:100 +	1:120 +	0	0	0	0

— = sterile; + = growth; controls showed heavy growths.

These experiments have shown that mercurophen in 1:5,000 dilution and even as high as 1:10,000 can be relied on for the sterilization of such instruments as cannot be sterilized with heat, with an exposure of at least 5 minutes; furthermore, the objectionable deleterious influence of mercuric chlorid on nickel plated and steel instruments with tarnishing, is not true of mercurophen inasmuch as the latter substance does not tarnish metals (Fig. 3).

THE NONTARNISHING EFFECT OF MERCUROPHEN ON INSTRUMENTS

Experiments concerning the influence of mercurophen and other mercurial compounds on surgical instruments have been conducted by immersing steel, nickel and silver-plated instruments in 1 : 1,000 solutions for periods of 24-48 hours at room temperature. Of the mercurial compounds tested in this manner only mercurophen and mercury succinimid did not tarnish any of these instruments to the slighest degree, whereas mercuric chlorid, mercury acetate, mercury sulphate, mercury cacodylate and mercury oxycyanid produced considerable

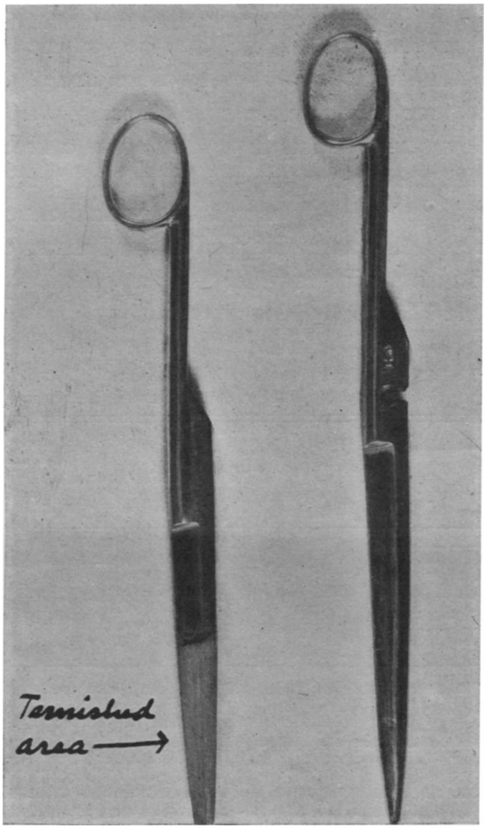

Fig. 3.—The tarnishing of a nickel-plated instrument (left) by mercuric chlorid in dilution of 1: 1,000; absence of tarnishing of similar instrument by mercurophen in dilution of 1: 1,000 (right).

tarnishing particularly of steel but not of silver-plated instruments (Fig. 3); as is well known, mercuric chlorid cannot be used for the disinfection of surgical instruments by reason of this deleterious influence, as this compound not only tarnishes steel and nickel-plated instruments but may even discolor silver-plated instruments on prolonged exposure.

The question of tarnishing does not depend on the percentage of mercury in a compound (table 22), but rather on the ease with which mercury is precipitated out of solution by nickel and steel; for example, mercuric chlorid, mercury acetate and mercury sulphate are compounds in which mercury exists in a solution as a free ion and there compounds tarnish metals and are most likely to precipitate proteins. Of the compounds tested, only mercurophen and mercury succinimid were found free of this tarnishing effect on instruments and either may be used with impunity for the disinfection of instruments in as low dilution as 1:1,000.

TABLE 22

THE INFLUENCE OF 1:1,000 SOLUTIONS OF MERCUROPHEN AND OTHER MERCURIAL COMPOUNDS ON THE METAL OF SURGICAL INSTRUMENTS

Substance	Percentage Pure Hg	Nature of Compound	Results	
			Silver Plated	Steel and Nickel Plated
Mercurophen	53	Half complex organic salt	—	—
Hg succinimid	50	Pseudocomplex organic salt	—	—
Hg oxycanid	83	Organic salt	—	+
Hg cacodylate	20	Organic salt	—	+
Hg acetate	63	Organic salt	—	+
Hg sulphate	67	Inorganic salt	—	+
Mercuric chlorid	74	Inorganic salt	+	+

Results after 48 hours exposure at room temperature; — = no effect; + = tarnishing.

Disinfection of the Skin.—In these experiments both hands of a series of laboratory assistants were cultured without previous washing, by rigorous rubbing of the skin in various parts with sterile swabs moistened with sterile broth or removal of epidermis with sterile scalpels followed by inoculation of tubes of glucose broth and 48 hours' incubation. As expected, all of these cultures showed heavy growths of various bacteria in which staphylococci and B. subtilis predominated. Immediately after the preliminary cultures were made the left hand was completely immersed in a sterile basin containing the disinfectant solution at 40 C. and the right hand in a similar basin containing sterile normal salt solution at the same temperature as a control.

After varying intervals of exposure to disinfectant the left hand was flushed with large volumes of sterile water at 40 C. to remove the disinfectant solution and both hands again cultured with particular attention to the finger nails and the skin between the fingers. The right or control hand invariably showed growths after the longest period of immersion in the warm sterile salt solution; a summary of the results with mercurophen and mercuric chlorid in these experiments is given in table 23.

TABLE 23

RESULTS OF HAND DISINFECTION TESTS WITH MERCUROPHEN AND MERCURIC CHLORID

Substance	Dilution	Exposure and Results								
		Prelim.	1 Min.	3 Min.	5 Min.	7 Min.	10 Min.	12 Min.	15 Min.	25 Min.
Mercurophen No. 6	1:1,000	+	—	—	—	—	—	—	—	—
Mercurophen No. 6	1:5,000	+	+	—	—	—	—	—	—	—
Mercurophen No. 6	1:10,000	+	+	+	+	+	—	—	—	—
Mercurophen No. 6	1:40,000	+	+	+	+	+	+	+	—	—
Mercuric chlorid	1:1,000	+	+	+	—	—	—	—	—	—
Mercuric chlorid	1:5,000	+	+	+	+	—	—	—	—	—
Mercuric chlorid	1:10,000	+	+	+	+	+	+	+	+	—
Mercuric chlorid	1:40,000	+	+	+	+	+	+	+	+	+

+ = growth; — = sterile.

In some experiments cultures made of the hands after immersion in 1:40,000 mercurophen for this period of time, showed growth of bacteria.

As a general rule, a warm solution of mercurophen in strength of 1:1,000 disinfected the skin within 1 minute and 1:5,000 disinfected in from 1-3 minutes and mercuric chlorid in the same strength in from 5-7 minutes; in 1:10,000 dilution mercurophen required at least 10 minutes, and mercuric chlorid almost half an hour. In higher dilutions the results were more irregular; for example, mercurophen in dilution of 1:40,000 sometimes disinfected the skin after an exposure of 15 minutes or more, depending apparently on the condition of the skin, inasmuch as this factor was quite variable because the skin was not previously prepared. Mercuric chlorid invariably failed to disinfect the skin in these higher dilutions over a 30-minute period of exposure.

Similar experiments with the skin of hands after preliminary washing and scrubbing with a soft brush, green soap and hot water for a period of 5 minutes followed by rinsing in hot sterile water, yielded more irregular results inasmuch as the control hands were occasionally sterile and the action of mercurophen and mercuric chlorid more effective in higher dilutions or with shorter exposures. For use in operating rooms a hot solution of 1:5,000 mercurophen with a period of immersion of at least 1 minute following the usual preliminary cleaning, can be recommended on the basis of these experiments.

The Nonirritant Properties of Mercurophen on the Skin.—In this connection emphasis may be placed on several observations indicating that solutions of mercurophen do not produce the dermatitis with which some persons suffer following the use of the ordinary mercurial compounds and particularly mercuric chlorid. Three physicians who suffer with a mercurial dermatitis following contact with solutions of mercuric chlorid, have voluntarily acquainted us with these observations and are able to use mercurophen in dilutions as low as 1:2,000 with impunity.

On the Eye.—In order to test the comparative irritant properties of mercurophen, particularly in view of the findings of several ophthalmologists indicating that mercurophen has remarkable curative properties in pneumococcus conjunctivitis, solutions of different strength have been dropped into the eyes of rabbits. These experiments were conducted by daily instillation of mercurophen solution into the right eye and an equal amount of a similar solution of mercuric chlorid into the left eye of each animal as a control. With 1:100 solutions of mercuric chlorid a severe purulent conjunctivitis was produced after 1 or 2 instillations, whereas after 3 and 4 instillations with 1:100 mercurophen but slight congestion was produced (Figs. 4 and 5); with 1:1,000 solutions, mercurophen produced no visible effects after 7 successive instillations, whereas mercuric chlorid produced slight conjunctivitis with erythema and slight discharge.

Solutions of mercurophen in strength of 1:1,000 and higher have also been used in the treatment of infantile gonococcus vaginitis by daily instillation into the vagina, with no evidences of irritation of the vaginal mucosa and introitus.

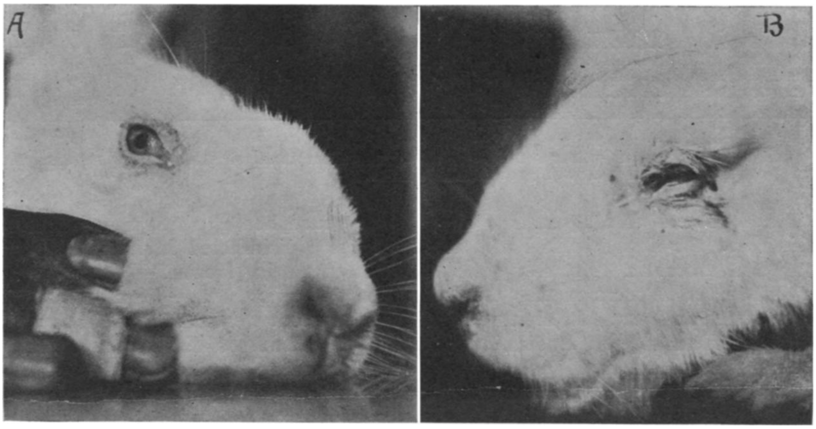

Fig. 4.—*A*, Eye of rabbit after three instillations of 2 drops of mercurophen in dilution of 1:100; mild hyperemia of conjunctiva.

B, Eye of rabbit after two instillations of 2 drops of mercuric chlorid in dilution of 1:100; severe purulent conjunctivitis.

These experiences have shown that mercurophen is remarkably free of irritant effects and that it may be used in contact with the skin and mucous membranes in dilutions as concentrated as 1:1,000 without apparent harm.

The Disinfection of Pus.—The results of these experiments varied according to the consistency of the pus, degree of phagocytosis and the type of infecting micro-organisms. In one experiment pus from an empyema which proved sterile was contaminated by adding 1 c c of a 24-hour broth culture of Staph. aureus to each 9 c c of pus and incubating several hours. Cultures of this material yielded heavy growths of staphylococci. Equal parts of this pus and varying dilutions of the disinfectants were well mixed, kept at room temperature and subcultured at intervals by transferring a 4 mm. loopful to glucose agar. The results of this experiment are shown in table 24; mercurophen in final dilution of 1:200 sterilized the pus in 5 minutes and in 1:5,000 dilution in 30 minutes. Mercuric chlorid in final dilution of 1:2,000 required 15 minutes and in 1:5,000 dilution failed in the longest period of exposure, namely, 30 minutes; phenol in final dilution of 1:40 required 5 minutes and in 1:80 required 30 minutes for complete disinfection.

TABLE 24

GERMICIDAL ACTIVITY OF MERCUROPHEN, MERCURIC CHLORID AND PHENOL IN STAPHYLOCOCCUS INFECTED PUS

Substance	Final Dilution	Results after Exposure for Minutes				
		1	5	10	15	30
Mercurophen........................	1:1,000	—	—	—	—	—
Mercurophen........................	1:2,000	+	—	—	—	—
Mercurophen........................	1:5,000	+	+	+	+	—
Mercuric chlorid....................	1:1,000	—	—	—	—	—
Mercuric chlorid....................	1:2,000	+	+	+	—	—
Mercuric chlorid....................	1:5,000	+	+	+	+	+
Phenol..............................	1:40	+	—	—	—	—
Phenol..............................	1:80	+	+	+	+	—
Control.............................	....	+	+	+	+	+

— = sterile; + = growth.

In a second experiment employing pus of an empyema containing large numbers of a virulent streptococcus with some phagocytosis, 0.5 c c of pus was placed in each of a series of large sterile test tubes with 0.5 c c of varying dilutions of disinfectant; after standing for 15 minutes at room temperature 10 c c of serum glucose agar at 45 C. was added to each tube, mixed and poured in sterile petri dishes following by incubation for 4 days. The results showed complete sterilization by mercurophen in final dilution as high as 1:12,000 and mercuric chlorid in dilutions to 1:3,200; phenol was not employed.

In this connection it may be stated that experiments with varying dilutions of mercurophen and other mercurial compounds with emulsions of guinea-pig spleen, kidney and liver, have shown the entire absence of solvent action such as occurs with Dakin's solution and allied chlorin compounds.

TABLE 25

THE GERMICIDAL ACTIVITY OF MERCUROPHENIZED GAUZE FOR STAPHYLOCOCCUS AUREUS

Substance	Gauze Impregnated with Dilution	Results after Exposure for Minutes			
		1	5	15	30
Mercurophen............................	1:1,000	—	—	—	—
Mercurophen............................	1:5,000	—	—	—	—
Mercurophen............................	1:10,000	+	+	—	—
Mercuric chlorid........................	1:1,000	—	—	—	—
Mercuric chlorid........................	1:5,000	+	+	+	—
Mercuric chlorid........................	1:10,000	+	+	+	+

— = sterile; + = growth.

The Germicidal Activity of Mercurophenized Gauze.—Ordinary surgical gauze was thoroughly saturated with 1:1,000, 1:5,000 and 1:10,000 solutions of mercurophen and similar dilutions of mercuric chlorid and dried at 50 C. Small squares measuring about 1 inch and of 4 thicknesses were placed for 1 minute in a 24-hour broth culture of Staph. aureus, the excess of broth removed by gentle squeezing and each portion so treated placed in separate sterile petri dishes. At varying intervals these squares of gauze were planted in flasks containing 500 c c of nutrient broth in order to highly dilute the disinfectant carried over, and incubated for 4 days. The results of an experiment are shown in table 25; gauze impregnated with a 1 : 5,000 dilution of mercurophen proved completely germicidal after 1 minute of contact with the cocci and in 1 : 10,000 dilution proved germicidal within 15 minutes. Gauze impregnated with a 1 : 5,000 solution of mercuric chlorid required about 30 minutes of contact to destroy all of the cocci and the 1 : 10,000 gauze failed to sterilize in the 30-minute period of exposure.

Similar experiments with pus yielded irregular results according to the density of the pus, the degree of phagocytosis and the thickness of the layer of pus in contact with the gauze. The experiments have shown, however, that, gauze impregnated with a 1 : 500 solution of mercurophen is strongly germicidal and likely to prove useful in the dressing of wounds when a nonirritant disinfectant will aid in reducing bacterial activity.

THE TOXICITY OF MERCUROPHEN

The toxicity of mercurophen has been studied by determining the highest tolerated dose for white rats by intravenous injection and for white mice by intraperitoneal injection, over a period of 10 days.

TABLE 26

Results of Toxicity Tests with Mercurophen; Intravenous Injection into Rats

Weight in Grams	Dose per Kilo	Amount of 1:1,000 Solution Injected in C C	Time of Injection in Seconds	Results in Days						
				1	2	3	4	5	6	10
235	0.02	0.7 (1:100)	84	D						
220	0.01	2.2	264	—	—	—	—	—	—	D
95	0.009	0.9	106	—	—	D				
90	0.008	0.8	96	—	—	—	—	—	—	—
95	0.007	0.7	84	—	—	D				
165	0.006	1.0	120	—	—	—	—	—	—	—
130	0.005	0.65	78	—	—	—	—	—	—	—
85	0.004	0.3	36	—	—	—	—	—	—	—

In this experiment the mercurophen contained 53% of mercury.

TABLE 27

Results of Toxicity Tests with Mercuric Chlorid; Intravenous Injection into Rats

Weight in Grams	Dose per Kilo	Amount of 1:1,000 Solution Injected in C C	Time of Injection in Seconds	Results in Days						
				1	2	3	4	5	6	10
105	0.010	1.1	132	D						
120	0.008	0.96	120		D					
110	0.007	0.77	92	—	D					
120	0.006	0.72	86	—	—	D				
150	0.005	0.75	90	—	—	—	D			
150	0.004	0.6	72	—	—	—	—	—	—	D
150	0.003	0.45	54	—	—	—	—	—	—	—
100	0.002	0.2	60	—	—	—	—	—	—	—

The mercuric chlorid contained 74% mercury.

Dosis Tolerata by Intravenous Injection.—In these experiments white rats weighing from 100-200 gm. were employed; each animal was weighed and injected with 1:1,000 solutions in water in doses varying from 0.002-0.02 gm. per 1000 gm. of weight. The injections were made into the saphenous vein by gravity and at the rate of about 0.5 c c of solution per minute. All animals were kept under observation for a period of 10 days.

For comparative results other compounds containing varying amounts of mercury, as mercuric chlorid, mercury succinimid and mercury cacodylate were injected into rats in the same manner.

The results of several experiments are shown in tables 26, 27, 28, 29 and 30; as is the general rule in such experiments irregular deaths have occurred rendering multiple tests necessary in order to arrive at general conclusions.

TABLE 28

RESULTS OF TOXICITY TESTS WITH MERCURY CACODYLATE; INTRAVENOUS INJECTION INTO RATS

Weight in Grams	Dose per Kilo	Amount of 1:1,000 Solution Injected in C C	Time of Injection in Seconds	Results in Days						
				1	2	3	4	5	6	10
100	0.02	2.0	240	—	—	—	D			
115	0.01	1.6	192	—	—	—	—	D		
105	0.009	0.95	120	—	—	D				
120	0.008	0.96	120	—	—	—	—	—	—	—
105	0.007	0.74	89	—	—	—	—	—	—	—
85	0.006	0.51	62	—	—	—	—	—	—	—

The cacodylate contained 20% mercury.

TABLE 29

RESULTS OF TOXICITY WITH MERCURY SUCCINIMID; INTRAVENOUS INJECTION INTO RATS

Weight in Grams	Dose per Kilo	Amount of 1:1,000 Solution Injected in C C	Time of Injection in Seconds	Results in Days						
				1	2	3	4	5	6	10
145	0.01	1.45	174	—	—	—	D			
175	0.008	1.4	168	—	—	D				
170	0.007	1.2	144	—	—	—	D			
120	0.006	0.7	84	—	—	—	—	—	—	—
80	0.005	0.4	48	—	—	D				
95	0.004	0.38	45	—	—	—	—	—	—	—
90	0.002	0.18	24	—	—	—	—	—	—	—

The succinimid contained 50% mercury.

With the method of injection employed, namely, slow administration from a graduated pipet by gravity with a very satisfactory apparatus designed by Dr. G. C. Lake of the Hygienic Laboratory of the U. S. Public Health Service for testing the toxicity of arsphenamin, the highest tolerated dose of mercurophen was about 0.008 gm. per kg. of body weight; mercuric chlorid was about 0.003-0.004 gm.; the succinimid of mercury about 0.006 gm. and the cacodylate of mercury about 0.008 gm. per kilogram of weight.

In our former studies on the comparative toxicity of mercurial compounds,[4] the intravenous injections into rats were made with a syringe into the jugular vein, the injection being quite rapid and not infrequently made in a few seconds, the dose per rat being dissolved in exactly 1 c c of water. With this technic the dosis tolerata of mercuric chlorid was from 0.002-0.004 gm. per kg. of body weight, usually being nearer the 0.002 gm. dose; the dosis tolerata of the succinimid was about 0.002 gm. and the cacodylate of mercury averaged 0.004-0.005 gm. per kg. of weight. The slower method of injection employed in the present series of experiments has, therefore, increased the dosis tolerata of these mercurial compounds in an appreciable manner.

Dosis Tolerata by Intraperitoneal Injection.—With intraperitoneal injection in white mice, each dose was given according to the body weight dissolved in 1 c c of water. As shown in table 30, mercurophen was tolerated in doses as high as 0.02 gm. per kg. of body weight while with mercuric chlorid the tolerated dose was about 0.005 gm. per kg. of weight.

TABLE 30

THE TOXICITY OF MERCUROPHEN AND MERCURIC CHLORID; INTRAPERITONEAL INJECTIONS IN MICE

Dose per Kilogram of Weight	Mercurophen						Mercuric Chlorid, Days					
	1	2	3	4	5	10	1	2	3	4	5	10
0.04	D						D					
0.035	D						D					
0.03	—	D					D					
0.025	—	—	—	—	D		D					
0.02	—	—	—	—	—	—	D					
0.015	—	—	—	—	—	—	D					
0.01	—	—	—	—	—	—	—	D				
0.008	—	—	—	—	—	—	—	—	—	D		
0.006	—	—	—	—	—	—	—	—	—	—	—	D
0.005	—	—	—	—	—	—	—	—	—	—	—	—

In our former studies we showed that the toxicity of mercurial compounds was, in general, directly proportionate to the amount of mercury contained and that on this basis of calculation the inorganic salts, as represented by the bichlorid of mercury, were no more toxic than the pseudo-complex organic combinations as represented by the succinimid and cacodylate. For this reason mercurophen with 53% mercury was not expected to be as toxic as mercuric chlorid with 74% mercury, but allowing for these differences in content of pure mercury, mercurophen is less toxic than its content in mercury would lead one to expect; for example, the succinimid of mercury used in this study contained 50% mercury but was distinctly more toxic than mercurophen containing 53% of mercury, inasmuch as the tolerated dose of the former by intravenous injection was 0.002-0.004 gm. per kg. of body weight as compared with 0.006 gm. of the latter compound. The reduction of toxicity of mercurial compounds without parallel reduction in mercury content and anti-parasiticidal activity constitutes one of the chief desiderata in the experimental chemotherapy of these compounds, and the beginning appears to have been attained in sodium oxymercury orthonitro phenolate.

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[4] Schamberg, J. F.: Kolmer, J. A., and Raiziss, G. W.: Boston Med. and Surg. Jour., 1915, 162, p. 826.

SPIROCHETICIDAL AND TRYPANOCIDAL ACTIVITY OF MERCUROPHEN

Spirocheticidal Activity. — The spirocheticidal activity of mercurophen has been tested by the intravenous and intramuscular administration of varying doses to rabbits with well developed syphilitic orchitis; in all instances 1:1,000 solutions in water were employed and the doses arranged according to the body weight of each animal.

As a general rule the intravenous injection of mercurophen in the dose of 0.0006 gm. per kilogram of body weight for 1 or 2 doses is followed by the disappearance of all spirochetes in the testicular lesions within 3-8 days after treatment began, followed by prompt healing of the syphiloma; with 1 or 2 intramuscular injections in amounts of 0.002 gm. per kg. of body weight the spirochetes are likewise destroyed and the lesions heal, but more slowly than is observed following intravenous medication. Our experiments have shown that mercurophen possesses spirochetical properties, but we have not conducted a sufficient number of comparative tests with other mercurial compounds to permit a statement being made of the relative value of mercurophen in the treatment of syphilitic injections. One of us (Schamberg) has injected mercurophen suspended in oil intramuscularly in several persons suffering with syphilis, but the local reaction was too severe to permit the continued administration of the drug by this route.

Trypanocidal Activity.—Experiments on the trypanocidal activity of mercurophen were conducted after methods previously described by us[5] and consisting briefly in the injection of white rats with intraperitoneal injections of approximately known numbers T. equiperdum after the method of Kolmer,[6] followed 24 hours later by the intravenous injection of graded doses of the drug dissolved in water per 100 gm. of body weight of each animal. In each experiment at least 4 control rats were included, receiving the infection alone but no drug; the strain of trypanosomes employed was of such virulence that the parasites appeared in the peripheral blood within 48 hours after infection and killed on or about the 7th day with tremendous numbers of trypanosomes in the blood. In conducting the experiments the blood of each animal was examined daily for trypanosomes and the results recorded according to the number present in a drop of blood from the tail.

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[5] Schamberg, J. F., Kolmer, J. A., and Raiziss, G. W.: Jour. Am. Med. Assn., 1915, 65, p. 2142.

[6] Jour. Infec. Dis., 1915, 17, p. 79.

The results of one experiment with mercurophen and mercuric chlorid shown in table 31, are fairly representative of those observed in a large series of similar experiments described by us in a former communication;[7] at best the maximum sublethal doses of mercurophen inhibited the multiplication of trypanosomes for 24 hours, but did not sterilize the animals.

TABLE 31

INFLUENCE OF MERCUROPHEN AND MERCURIC CHLORID ADMINISTERED INTRAVENOUSLY 24 HOURS AFTER INFECTION WITH T. EQUIPERDUM

Weight of Rat, Gm.	Dose per 100 Gm.	Compound	Daily Examination					
			1	2	3	4	5	6
100	0.0008	Mercurophen	—	—	D			
90	0.0007	Mercurophen	—	few	+	++	D	
95	0.0006	Mercurophen	—	few	++	++++	D	
110	0.0005	Mercurophen	—	few	++	++++	D	
120	0.0008	Mercuric chlorid	—	few	D			
115	0.0007	Mercuric chlorid	—	few	++	+++	D	
90	0.0006	Mercuric chlorid	—	few	++	+++	D	
100	0.0005	Mercuric chlorid	—	few	++	++++	++++	D
90	0	Control	—	few	++	++++	D	
120	0	Control	—	few	++	++++	++++	D

Rats infected with 110,000 trypanosomes by intraperitoneal injection.

Inasmuch as these tests were very severe by reason of employing a virulent strain of trypanosomes given a 24 hours start, further experiments were conducted in which the drugs were administered intravenously 2 hours after infection; in these tests mercurophen showed varying degrees of inhibition of multiplication of trypanosomes, in one instance the blood being kept free of parasites for 4 days.

TABLE 32

INFLUENCE OF MERCUROPHEN AND MERCURIC CHLORID ON T. EQUIPERDUM IN THE COMBINED TEST IN VITRO-VIVO

Weight of Rats in Gm.	Dose in Gm.	Compound	Results of Daily Examination of Blood from the Tail															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
92	0.0005	Mercurophen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
90	0.00025	Mercurophen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
115	0.000125	Mercurophen	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
105	0.000062	Mercurophen	—	—	—	—	—	—	—	—	—	—	—	few	+	4+	4+	D
97	0.0005	Mercuric chlorid	—	—	—	—	—	—	—	—	—	—	—	—	D			
115	0.00025	Mercuric chlorid	—	—	+	3+	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
65	0.000125	Mercuric chlorid	—	—	+	4+	D											
80	0.000062	Mercuric chlorid	—	—	+	2+	2+	2+	2+	4+	D							
105	0	Control	—	—	+	4+	D											
90	0	Control	—	—	+	2+	D											

In additional experiments employing a combined in vitro-vivo technic, in which equal parts of varying dilutions of the chemical under study were mixed with blood-trypanosome emulsion and kept at 37-40 C. for fifteen minutes, when the whole or a part was injected intraperitoneally into white rats to determine the degree of trypanocidal activity, mercurophen and several other mercurial compounds were found to exercise definite trypanocidal activities as shown in the results of one experiment in table 32.

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[7] Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: Am. Jour. Syphilis, 1917, 1, p. 1.

The conclusions drawn by us from these experiments are to the effect that mercurophen and other mercurial compounds as mercuric chlorid, possess some trypanocidal activity, but to a lesser degree than that exhibited by arsphenamin administered to infected rats in equal dosage per body weight.

BACTERICIDAL ACTIVITY OF MERCUROPHEN IN VIVO

Owing to the high germicidal activity of mercurophen in vitro in solutions containing large amounts of protein as 50% dilutions of oxalated blood and serum, we have conducted a series of experiments in rabbits to determine if a sufficient quantity of the drug could be administered intravenously to protect the animal against infection with such micro-organisms as virulent pneumococci and staphylococci, or at least impart such increased bactericidal activity to the blood as could be detected by the withdrawal of blood and the subjection of the same to bactericidal tests in vitro.*

Experiments with Pneumococci.—The virulence of a type I strain of pneumococcus for young white rats weighing from 55-90 gm. was determined by injecting various doses of 24-hour blood dextrose broth cultures intraperitoneally and intramuscularly; the minimum lethal doses of the culture by each route of infection 3 days later were as follows:

Intraperitoneal injection: 0.001 c c.
Intramuscular injection: 0.01 c c.

A. A series of young rats weighing from 55-70 gm. were injected intravenously with mercurophen in dose of 0.001 gm. per kilogram of body weight 2 hours before, simultaneously with and 2 hours after infection produced by the intraperitoneal injection of 10 M. L. D's. of the culture previously determined for this route of infection. Controls received drug and culture alone.

The animals receiving culture alone died on the 3rd and 4th days; those receiving the drug alone lived indefinitely. Those receiving drug and pneumococci died on or before the 3rd day, mercurophen in the dosage mentioned affording no protective or curative action.

B. A second series of rats of the same range in weight were injected intravenously with mercurophen in dose of 0.001 gm. per kilogram of body weight 2 hours before, simultaneously with and 2 hours after infection produced with the intramuscular injection of 10 M. L. D's. of the culture, in order to bring about a more slowly developing infection.

The controls receiving the culture alone died 3-4 days later; those receiving drug alone lived indefinitely. Those receiving drug and pneumococci died on the 2nd and 3rd days, mercurophen failing to afford protection and indeed, appearing to hasten death probably by the toxic influence of the drug added to the virulence of the culture.

Experiments with Staphylococci.—The virulence of a strain of Staph. aureus for white rats weighing about 90 gm. was determined by injecting increasing amounts of a filtered 24-hour broth culture intravenously, necropsying all animals 3 days later and examining the viscera for abscesses. The smallest dose of the culture employed regularly producing abscesses in the kidneys, heart and diaphragm was 0.5 c c; in conducting the main experiments the culture was employed in twice this amount, or 1 c c.

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* These experiments were conducted with the assistance of Dr. Edward Steinfield.

The main experiments were conducted by injecting mercurophen intravenously in doses of 0.001 gm. per kg. of body weight, at the following intervals:

Two hours before infection, simultaneously with infection, 2, 4, 6 and 24 hours after infection.

As previously stated the animals were infected by injecting them intravenously with 1 c c of culture. Controls received culture alone and all developed numerous abscesses in the viscera and particularly the kidneys; controls receiving drug alone showed no changes at necropsy.

All the animals receiving mercurophen and the staphylococci after the plan given above showed abscesses in the kidneys and other organs when examined 3 days later; the number and size of these abscesses varied in the different animals but bore no relation to the administration of the drug, all experiments indicating that mercurophen in 1 dose of 0.001 gm. per kg. (equivalent to 0.06 gm. or 1 grain per 60 kg.) administered intravenously exerts no protective or curative action.

Blood Cultures.—Additional experiments were conducted with rabbits consisting in making preliminary blood cultures with 5 drops of blood from each animal in tubes of dextrose broth (10 c c) followed by the intravenous injection of 24-hour filtered broth cultures of Stap. aureus in varying doses, namely, 0.5, 1 and 2 c c and 10 minutes later by intravenous injection of mercurophen in dose of 0.001 gm. per kg. of body weight; a second series of animals received 0.0015 gm. mercurophen per kg. of body weight. The controls received culture alone and mercurophen alone (0.0015 gm. per kg). Blood cultures were repeated at intervals of 10 minutes, 1, 4, 24 and 48 hours; and all yielded positive cultures for staphylococci and all infected animals showed a variable number of abscesses in the kidneys on the 3rd day of the experiments.

The influence of multiple doses of mercurophen has not been tried; it is possible that in such experiments some influence may be exerted inasmuch as the following experiments show that an increased bactericidal activity of the blood may be noted soon after the administration of mercurophen, which disappears within a few hours.

Bactericidal Activity of Rabbit Serum Following the Intravenous Administration of Mercurophen.—In these experiments small amounts of blood were collected from the ear veins of rabbits and mercurophen injected intravenously followed by further bleedings at varying intervals up to 24 hours. The serums were then separated, heated at 56 C. for one-half hour and mixed in sterile test tubes in amounts of 0.2 c c with an equal quantity of a diluted 24-hour broth culture of Staph. aureus. After 5 hours' incubation at 37 C. the contents of each test tube were plated with 10 c c of dextrose agar at 45 C. and the plates counted after 48 hours' incubation.

The results of these experiments indicated that large doses of mercurophen imparted an increased bactericidal activity to the serum which usually had disappeared within a few hours and always at the end of 24 hours. In order to detect this increase of bactericidal activity of the blood it was necessary to use doses of culture sufficiently small to produce a countable number of colonies when plated with agar. The results of an experiment shown in table 33 is representative of those usually observed; the greatest bactericidal activity was noted with serum collected within an hour after the administration of the mercurophen; with larger doses of culture resulting in the production of an uncountable number of colonies, this bactericidal activity was masked.

TABLE 33

BACTERICIDAL ACTIVITY OF RABBIT SERUM FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MERCUROPHEN

Dose of Mercurophen per Kg.	Dose of Heated Serum in C C	Dose of Culture of Staphylococci	Results of Plating			
			Before Injection	1 Hour after Injection	4 Hours after Injection	24 Hours after Injection
0.0015	0.2	0.2 c c (1:100,000)	81,000	180	205	70,000
0.0015	0.2	0.2 c c (1:10,000)	unc.	unc.	unc.	unc.

Further experiments were conducted with a multiple pipet method modified after the technic devised by Heist and Lacy and described by Heist, Cohen and Cohen[8] for the detection of natural antibodies in the whole blood of persons and animals for pneumococci and the globoid bodies of poliomyelitis. With this technic, mercurophen, mercuric chlorid and other compounds were administered to rabbits intravenously and the bactericidal activity of the blood studied with the many stemmed pipet for staphylococci, pneumocicci and B. typhosus. A description of the technic employed and a detailed account of the experiments are to be given elsewhere;[9] here it may be stated that following the intravenous injection of mercurophen in dose or 0.0015 gm. per kg. of body weight, the whole uncoagulated blood was found to have an increased bactericidal activity for pneumococci, staphylcocci and B. typhosus over a period of 1-2 hours following the injection: similar experiments with mercuric chlorid and other mercurial compounds usually failed to increase to the same degree the bactericidal activity of the uncoagulated blood of rabbits for these micro-organisms.

Influence of Mercurophen on the Total Number of Leukocytes in the Blood.—During the course of several experiments among rabbits in which mercurophen alone was administered intravenously in varying doses per kg. of body weight, total and differential leukocytic counts were made of the blood of a number of animals to determine what influence, if any, was excited by the drug. The results of a few counts made at varying intervals after the administration of mercurophen, are shown in table 34; as a general rule, the administration of mercurophen in dose of 0.0005-0.002 gm. per kg. of body weight in proportion of 0.001 gm in 1 c c of water, produced a slight leukocytosis with an increase of polymorphonuclear cells toward the end of the first 24 hours following the injection, with entire subsidence within 48 hours after injection.

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[8] Jour. Immunology, 1918, 3, p. 261.

[9] Kolmer, J. A., and Trist, M. E.: A Modification of the Heist-Lacy Method for Determining the Bactericidal Action of Whole Blood in Chemotherapeutic Studies.

TABLE 34

INFLUENCE OF MERCUROPHEN ADMINISTERED INTRAVENOUSLY ON THE LEUKOCYTES OF THE BLOOD

Rabbits	Weight in Gm.	Dose per Kilo in C C	Total Leukocyte Counts				
			Before Injection	1 Hour after Injection	4 Hours after Injection	24 Hours after Injection	48 Hours after Injection
1	1500	0.0005	7,800	9,600	9,200	10,600	12.800
2	1850	0.001	8.400	8.200	8.600	11,400	11,800
3	1620	0.0015	6,800	8,400	9,100	12,200	10,800
4	1700	0.002	6,200	7.200	7,600	9,800	11,600
5	1630	0.001	8,200	7,000	12,800	14,600	9,200
6	1800	water alone	8,400	8,600	9,200	8,000	8,200

THE INFLUENCE OF MERCUROPHEN ON PHAGOCYTOSIS

Since solutions of mercurophen have been used by several ophthalmologists with marked success in the treatment of pneumococcus conjunctivitis and by several surgeons in the treatment of wounds, a question of paramount importance concerns the influence of mercurophen on the phagocytic activity of polymorphonuclear leukocytes.

Experiments bearing on this problem were conducted by mixing in small sterile test tubes equal volumes of varying dilutions of mercurophen in physiologic salt solution and an emulsion of Staph. aureus followed by incubation in a water-bath for 1 hour at 37 C. when a volume of leukocytes secured from the peripheral blood of normal persons was added; after mixing, the tubes were re-incubated for an hour and stained smears prepared. Of each preparation 100 polymorphonuclear cells were counted and the percentage of phagocytes ascertained; no attempt was made to count the number of cocci within the cells. Numerous controls in which physiologic salt solution was substituted for mercurophen, were included in each experiment.

TABLE 35

THE INFLUENCE OF MERCUROPHEN ON PHAGOCYTOSIS

Experiment	Percentage of Leukocytes Becoming Phagocytes in Varying Final Dilutions of Mercurophen									
	1: 2,000	1: 5,000	1: 8,000	1: 10,000	1: 16,000	1: 20,000	1: 50,000	1: 80,000	1: 100,000	Control
1	0.5	2	6	5	7	9	11	8	7	6
2	0.5	2	4	3	4	7	4	4	3	2

The results of two experiments are shown in table 35. With the leukocytes of one person about 6% were found to engulf the staphylococci employed; mercurophen in final dilutions up to 1:5,000 were found to inhibit phagocytosis but in higher dilutions this effect was not in evidence and some tendency toward increased phagocytosis was noted with dilutions 1:20,000 to 1:50,000. In the second experiment employing the leukocytes of a second person and a different culture, spontaneous phagocytosis occurred with about 2% of leukocytes and mercurophen excited a similar influence.

These and additional experiments have shown, therefore, that strong solutions of mercurophen (1:1,000 to 1:5,000) may inhibit phagocytosis by a destructive influence on the leukocytes (fragmentation of protoplasm and alteration in staining), whereas higher dilutions do not exert this destructive influence and appear to enhance phagocytosis to a slight degree.

TABLE 36

SUMMARY SHOWING RESULTS OF COMPARATIVE TESTS WITH MERCUROPHEN AND MERCURIC CHLORID

Tests	Comparative Results and Properties	
	Mercurophen	Mercuric Chlorid
Highest antiseptic dilution for B. typhosus	1:480,000	1:540,000
Highest antiseptic dilution for Staph. aureus	1:1,200,000	1:540,000
Highest antiseptic dilution for B. anthracis	1:1,400,000	1:280,000
Phenol coefficient in Hygienic Laboratory test	900 to 1300	840
Rapidity of germicidal activity for staphylococci	1:100,000 (1 hour)	1:100,000 (more than 2 hr.)
Rapidity of germicidal activity for typhoid bacilli	1:100 000 (15 minutes)	1:100,000 (60 minutes)
Stability of solutions	Stable	Stable
Germicidal activity in blood	1:2,000 (60 minutes)	1:2,000 (more than 2 hr.)
Germicidal activity in ascites fluid	1:4,000 (1 minute)	1:4,000 (more than 10 min.)
Germicidal activity in serum	1:10,000 (1 minute)	1:10,000 (more than 30 min.)
Precipitation of serum proteins	Not in 1:200	Positive in 1:1,000
Disinfection of typhoid urine	1:8,000 (5 minutes)	1:8,000 (30 minutes)
Disinfection of ordinary urine	1:2,000 (5 minutes)	1:2,000 (60 minutes)
Disinfection of feces	1:1,000 (10 minutes)	1:1,000 (more than 10 min.)
Disinfection of sputum	1:4,000 (few minutes)	1:4,000 (more than 30 min.)
Disinfection of rubber gloves	1:5,000 (1 minute)	1:5,000 (1 to 3 minutes)
Disinfection of catheters	1:5,000 (8 minutes)	1:8,000 (10 minutes)
Disinfection of instruments	1:10,000 (1 to 5 min.)	1:10,000 (more than 15 min.)
Tarnishing of instruments	Absent	Marked
Disinfection of skin	1:5,000 (1 to 3 min.)	1:5,000 (5 to 7 min.)
Disinfection of skin	1:1,000 (1 minute)	1:1,000 (5 minutes)
Irritant action of skin	Absent	May occur
Irritant action on eye	Very slight	Marked
Disinfection of pus	1:2,000 (5 minutes)	1:2,000 (15 minutes)
Germicidal activity of impregnated gauze	1:5,000 (1 minute)	1:5,000 (30 minutes)
Dosis tolerata by intravenous administration	0.008 gm. per kilo	0.003 to 0.004 gm. per kilo
Dosis tolerata by intraperitoneal administration	0.02 gm. per kilo	0.005 gm. per kilo
Spirocheticidal activity	Present	Present
Trypanocidal activity	Present in greater deg.	Present
Protection against staphylococcus infection	None	None
Protection against pneumococcus infection	None	None
Bactericidal action of blood after administration	Present in greater deg.	Practically absent
Influence of phagocytosis	Inhibitory up to 1:5,000	0
Influence of leukocytic counts	Slightly stimulating	0

SUMMARY

Throughout this study mercuric chlorid was employed for comparative tests with mercurophen by reason of the fact that the properties of the former are so well known and its being the most widely used of the available mercurial preparations. Since the technic of a disinfectant test exerts great influence on the results, we have attempted to simplify our experiments as much as possible, and in every instance the experiments with mercurophen were conducted simultaneously with mercuric chlorid and other mercurial compounds in order that the same technic might be applied to both and the results be strictly comparative.

The technic employed in the various tests and the results observed with mercurophen, mercuric chlorid, other mercurial compounds and phenol are given in the body of this communication; a summary is given in table 36 which shows at a glance the results of comparative tests with mercurophen containing 53% mercury and mercuric chlorid containing about 74% mercury.

CONCLUSIONS

Sodium oxymercury-orthonitrate-phenolate, for convenience designated by us as mercurophen, possess certain superior properties over other mercurial compounds, as follows:

In germicidal activity mercurophen is equal or superior to mercuric chlorid and other mercurial compounds containing more mercury; inasmuch as the germicidal activity of this class of compounds bears a relation to their content in mercury and the electrolytic dissociation of the mercury compound in watery solution, mercurophen is of special interest from the chemotherapeutic standpoint.

Mercurophen is generally more rapid in its germicidal activity than other mercurial compounds.

Mercurophen appears to possess a special destructive affinity for cocci and spore-forming bacilli.

Mercurophen has proven superior to mercuric chlorid in the disinfection of urine, feces, sputum, pus, catheters, instruments, rubber gloves and the skin.

Mercurophen maintains a higher degree of germicidal activity in blood serum than mercurial chlorid.

Mercurophen does not precipitate protein in as high concentration as 1:100; nor does it irritate the skin or tarnish surgical instruments.

Mercurophen is somewhat more trypanocidal than mercuric chlorid and is capable of temporarily raising the bactericidal action of the blood after intravenous administration.

In terms of mercury, mercurophen is less toxic for animals than other soluble mercurial compounds and this constitutes a fact of much importance in the chemotherapy of this class of compounds.